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Study of the Antibiotic Resistance Patterns of Urinary Tract Infections in Hospitalized Children in Mazandaran

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Abstract

Background: Identifying antibiotic resistance in uropathogenic pathogens is essential to avoid treatment errors and minimize treatment costs. Despite the clinical significance of Urinary tract infections (UTIs) in children, limited data are available on the antimicrobial resistance patterns of the causative pathogens, highlighting the need for continuous surveillance. Our aim was to determine the pattern of drug resistance in strains isolated from children with UTI.

Methods: This retrospective cross-sectional study was conducted from 2021 to 2023 at Bo Ali Sina Hospital in Sari, Iran. A total of 1502 positive urine culture samples from hospitalized children were included. Antimicrobial susceptibility testing was performed using the disk diffusion method, following Clinical and Laboratory Standards Institute (CLSI). guidelines Data analysis was carried out using Statistical Package for the Social Sciences (SPSS) software.

Results: Of the 1502 cases, 68.8% were female. The most frequently isolated pathogen was *Escherichia coli* (54.8%), followed by *Pseudomonas aeruginosa* (3.3%) and *Klebsiella* spp. (3.1%). Among Gram-positive bacteria, *Staphylococcus epidermidis* (9.0%) was the most prevalent. The highest susceptibility rates were observed for amikacin across *E. coli*, *Klebsiella,* and *Pseudomonas* isolates.

Conclusions: Based on local susceptibility patterns, antibiotics such as amikacin, nitrofurantoin, gentamicin, imipenem, ceftazidime, and ceftriaxone may be considered for empirical treatment of complicated UTIs in hospitalized children to improve their care. However, whenever possible, narrow-spectrum antibiotics should be prioritized. Appropriate antibiotic selection must rely on microbial identification and resistance profiling.

Key Words: Antibiotic resistance, Urinary tract infection, Uropathogenic bacteria.

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1- INTRODUCTION

Urinary tract infections (UTIs) are one of the most common bacterial infections , affecting 150 million people worldwide every year (1, 2). UTIs can lead to serious complications in women, infant boys, elderly men, individuals with underlying urinary tract disorders, and those requiring long-term urethral catheterization (3). The infection may affect either the upper or the lower urinary tract (4). Infections may involve either the upper or lower urinary tract, and in infants, symptoms are often nonspecific, with fever being the most common clinical sign in children under two years of age (4, 5). Although all children and adolescents are susceptible to UTIs, some have a higher risk of developing the condition. Neonates are at risk in the first few months of life. Under the age of 1 year, the prevalence of UTI is higher in boys than in girls, and after 1 year of age, UTI is more common in girls than in boys (6).

Escherichia coli (*E. coli*) causes the vast majority of UTIs, accounting for 80-90% of cases in children (7). Uropathogenic *E. coli* (UPEC) is thought to spread from the stool flora to the urinary tract and bladder (8). Other important microorganisms include *Proteus*, *Klebsiella*, and *Enterococcus* (9). Although some mild UTIs may resolve spontaneously, antibiotic therapy is generally required to alleviate symptoms and prevent complications such as upper tract involvement or renal scarring (10). Oral antibiotic therapy for 7 to 10 days is sufficient for uncomplicated cases that respond well to treatment. Hence, UTI-causing pathogens have become increasingly resistant to commonly used antibiotics, and their indiscriminate use in suspected UTI cases should be stopped (11).

The increase of drug resistance in recent years among pathogens, especially UTI-causing agents, is a big problem.This is the main reason for the emergence of resistant strains, especially multi-drug resistant (MDR) strains, the spread of resistant agents to sensitive strains, increasing treatment costs, treatment failure , and increased mortality and morbidity (10, 12). The increase of antimicrobial resistance among urinary system bacteria makes treatment approaches more complicated and is considered a threat to public health, requiring new treatments based on basic biological research (13). In light of these concerns, the present study aimed to investigate the antimicrobial resistance patterns of bacterial strains isolated from pediatric patients with UTIs at a referral hospital in Sari, northern Iran, during 2021–2023.

2- MATERIALS AND METHODS

2-1. Patients and Sampling

A total of 1502 positive urine culture samples from patients who presented to Bo Ali Sina Hospital in Sari between 2021 and 2023 were retrospectively analyzed. All non-repetitive positive urine cultures were considered, regardless of the patients' clinical signs or other laboratory findings. Clinical specimens were promptly transported to the hospital's microbiology laboratory, where direct microscopic examinations and culture tests were performed.

2-2. Bacterial Isolation and Identification Procedures

Urine cultures showing bacterial growth exceeding 10^5 colony-forming units per milliliter (CFU/mL) were considered positive (14). Bacterial identification was carried out using standard phenotypic methods, including Gram staining, assessment of colony morphology, and a series of biochemical tests. Depending on the suspected microorganism, the following biochemical assays were employed: catalase, oxidase, and coagulase tests; Triple Sugar Iron

(TSI) agar; Simmons' citrate utilization; urease test; cultures on SIM medium for assessments of indole production; motility testing and hydrogen sulfide (H_2S) production; methyl red and Voges-Proskauer (MR-VP) tests; lysine decarboxylase; mannitol salt agar; DNase agar; bile-esculin hydrolysis; and various sugar fermentation tests (15).

2-3. Antimicrobial Susceptibility Test

The antimicrobial susceptibility testing of urine cultures was conducted using the disk diffusion method, adhering to the guidelines set by the Clinical and Laboratory Standards Institute (CLSI). The antibiotics tested included: amikacin (30 µg), amoxicillin (10 µg), co-amoxiclav (30 µg), ampicillin (10 µg), azithromycin (15 µg), cefalexin (30 µg), cefazolin (30 µg), cefixime (5 µg), cefotaxime (30 µg), cefoxitin (30 µg), ceftazidime (30 µg), ceftizoxime (30 µg), ceftriaxone (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), clindamycin (2 µg), cloxacillin (1 µg), cotrimoxazole (25 µg), gentamicin (10 µg), imipenem (10 µg), meropenem (10 µg), nalidixic acid (30 µg), nitrofurantoin (300 µg), norfloxacin (10 µg), oxacillin (1 µg), penicillin (10 µg), and vancomycin (30 µg). Results were interpreted as sensitive (S), intermediate (I), or resistant (R), based on CLSI criteria (16). Reference strains for both gram-negative and/or gram-positive organisms (*E. coli* ATCC 25922 and *Staphylococcus aureus* 25923) were included in each daily testing run as quality controls.

2-4. Statistical Analysis

Statistical analysis conducted using with the Statistical Package for the Social Sciences (SPSS) for Windows, version 26.0. The normality of variable distribution was assessed using a histogram and either the Kolmogorov-Smirnov or Shapiro-Wilk test. Continuous variables were present as median and interquartile range (IQR) values when not normally distributed. Categorical variables were reported as numbers and percentages, and compared using the chi-square test.

3- RESULTS

1502 children referred to medical educational hospitals of Mazandaran University of Medical Sciences in 1400-1402 were suffering from UTI, and they were included in the study to examine the pattern of antibiotic resistance in them. Among the 1502 examined samples, 1034 (68.8%) were girls and 468 (31.2%) were boys. Only 1242 of them had their age accurately recorded, among them 125 were infants (under one month) (10.1%). Most of the participants were older than 5 years old (431, 34.7%) (Figure 1).

As shown in Figure 2, the highest frequency of Gram-negative uropathogenic bacteria was *E.coli* with 823 cases (54.8%), followed by *P. aeruginosa* (49, 3.27%), *Klebsiella* spp. (47, 3.13%), *P. mirabilis* (37, 2.47%), *Enterobacter* spp. (21, 1.40%), *Acinetobacter* spp. (4, 0.27%), and *Citrobacter* spp. (1, 0.07%).

We also examined the frequency of each Gram-positive bacterium in the positive culture samples of children with UTI. In our study, the most common bacteria were *S. epidermidis* (136, 9.05%), followed by *S. saprophyticus* (46, 2.93%), *S. aureus* (16, 0.80%), *S.viridans* (10, 0.67%), and *E. faecalis* (1, 0.07%).

Furthermore, we investigated the antibiotic resistance of positive samples. The highest sensitivity was observed in amikacin antibiotic with 99.20%, and the highest resistance was against nalidixic acid with 63.6% (Figure 3).

In Tables 1 and 2, we investigated the resistance of each antibiotic in the samples of female and male patients with positive cultures separately. In order to better evaluate the resistance and sensitivity of antibiotics, we evaluated each of them in two different genders. Considering that the prevalence of UTIs in females is much higher than in males, examining each of the two sexes alone can help in choosing treatment for different sexes. For example, when we examined the two sexes separately, the amikacin sensitivity increased in females (from 20.98% to 99.3%) and decreased in males (from 20.98% to 95.5%). The same thing happens for nitrofurantoin; it shows more sensitivity in the female gender, and in the female gender, it shows a higher percentage of sensitivity in the total sample. In our study, nalidixic acid shows the highest antibiotic resistance; this antibiotic also shows higher antibiotic resistance when examined alone in the female gender, while this is not the case in the male gender and it shows a lower antibiotic resistance.

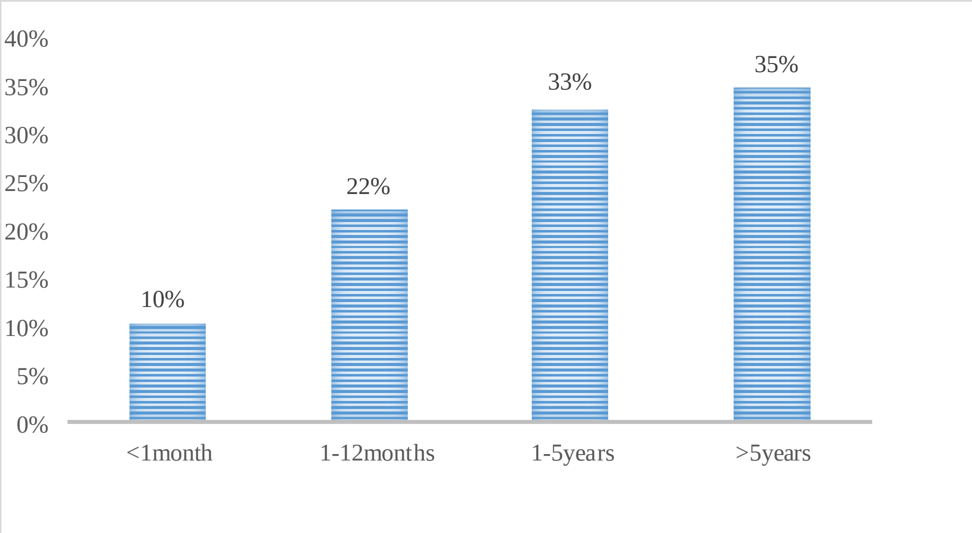

Figure-1: Age distribution of patients with urinary tract infections.

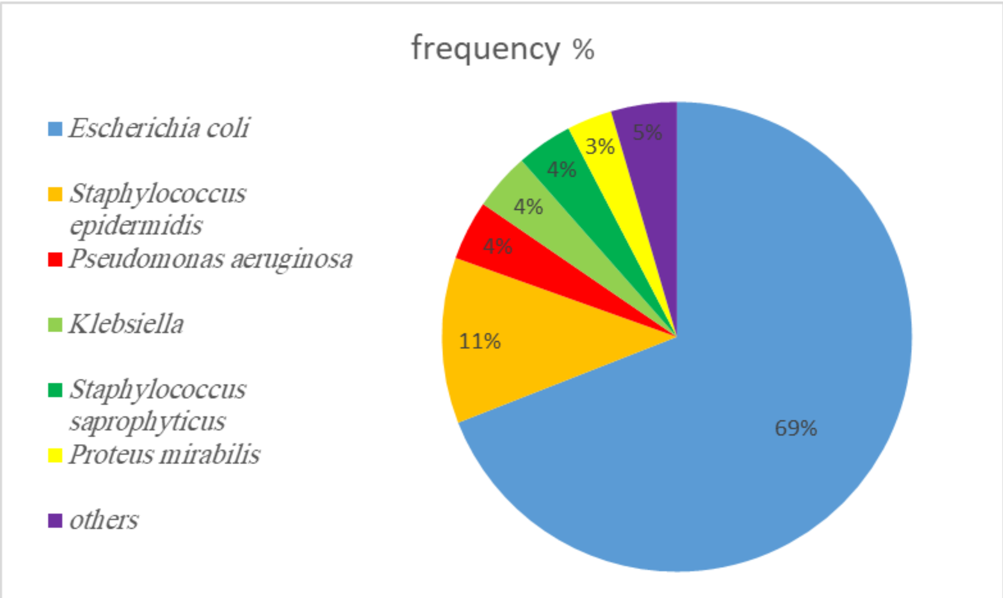

Figure-2: Distribution of the uropathogenic bacteria. Others: *Enterobacter* (21), *Acinetobacter* (4), *Staphylococcus aureus* (16), *Streptococcus viridans* (10), *Enterococcus faecalis* (1), *Citrobacter* (1), and *Streptococcus betahemolytic B* (1).

Table-1. Antibiotic resistance pattern in girls with urinary tract infections.

Percentage	Frequency	Resistance pattern	Antibiotic	Percentage	Frequency	Resistance pattern	Antibiotic
1.9	8	I	Ciprofloxacin	99.3	665	S	Amikacin
14.3	59	R		0.6	4	I	
83.8	346	S		0.1	1	R	
100	413	total		100	670	total	
1.8	1	I	Clindamycin	50	1	S	Amoxicillin
58.2	32	R		50	1	R	
40.0	22	S		100	2	total	
100	55	total		82.6	161	S	Co-amoxiclav
0	0	S	Cloxacillin	3.1	6	I	
0	0	total		14.4	28	R	
1.5	12	I	Co-trimoxazole	100	195	total	
42.3	332	R		36.6	237	S	Ampicillin
56.1	440	S		1.7	11	I	
100	784	total		61.7	399	R	
2.5	20	I	Gentamicin	100	647	total	
5.2	42	R		18.8	3	S	Azithromycin
92.3	748	S		18.8	3	I	
100	810	total		62.5	10	R	
2.7	1	I	Imipenem	100	16	total	
8.1	3	R		50.5	52	S	Cefalexin
89.2	33	S		1.9	2	I	
100	37	total		47.6	49	R	
0	0	S	Meropenem	100	103	total	
0	0	total		0.7	1	I	Cefazolin
35.3	6	S	Nalidixic acid	26.3	36	R	
64.7	11	R		73.0	100	S	
100	17	total		100	137	total	
1.3	11	I	Nitrofurantoin	3.1	2	I	Cefixime
4.4	36	R		31.3	20	R	
94.2	770	S		65.6	42	S	
100	817	total		100	64	total	
8.3	1	I	Norfloxacin	3.0	21	I	Cefotaxime
16.7	2	R		16.4	114	R	
75.0	9	S		80.6	560	S	
100	12	total		100	695	total	
1.4	1	I	Oxacillin	100	1	S	Cefoxitin
69.4	50	R		100	1	total	
29.2	21	S		3.3	20	I	Ceftazidime
100	72	total		8.5	52	R	
2.7	2	I	Penicillin	88.3	543	S	
29.3	22	R		100	615	total	
68.0	51	S		85.7	36	S	Ceftizoxime
100	75	total		14.3	6	R	
1.5	2	I	Vancomycin	100	42	total	
9.0	12	R		3.9	27	I	Ceftriaxone
89.5	119	S		13.5	92	R	
100	133	total		82.6	565	S	
				100	684	total	
				100	1	S	Chloramphenicol
				100	1	total	

Abbreviations; S: Sensitive, **I:** Intermediate, and **R:** Resistance.

Table-2. Antibiotic resistance pattern in boys with urinary tract infections.

Percentage	Frequency	Resistance pattern	Antibiotic	Percentage	Frequency	Resistance pattern	Antibiotic
2.1	3	I	Ciprofloxacin	1.2	3	I	Amikacin
15.2	22	R		3.3	8	R	
82.8	120	S		95.5	235	S	
100	145	total		100	246	total	
2.9	1	I	Clindamycin	0	0	S	Amoxicillin
54.3	19	R		0	0	R	
42.9	15	S		0	0	total	
100	35	total		3.3	2	I	Co-amoxiclav
100	1	S	Cloxacillin	21.3	13	R	
100	1	total		75.4	46	S	
1.5	12	I	Cotrimoxazole	100	61	total	
42.3	332	R		1.3	3	I	Ampicillin
56.1	440	S		60.7	142	R	
100	784	total		38.0	89	S	
3.5	11	I	Gentamicin	100	234	total	
11.1	35	R		0	0	I	Azithromycin
85.4	268	S		58.3	7	R	
100	314	total		41.7	5	S	
5.6	2	I	Imipenem	100	12	total	
5.6	2	R		2.5	1	I	Cefalexin
88.9	32	S		70.0	25	R	
100	36	total		27.5	11	S	
100	1	S	Meropenem	100	40	total	
100	1	total		0	0	I	Cefazolin
18.2	2	S	Nalidixic acid	31.7	13	R	
81.8	9	R		68.3	28	S	
100	11	total		100	41	total	
3.5	11	I	Nitrofurantoin	11.1	2	I	Cefixime
14.5	46	R		16.7	3	R	
82.0	260	S		72.2	13	S	
100	317	total		100	18	total	
0	0	I	Norfloxacin	3.5	10	I	Cefotaxime
28.6	2	R		28.4	81	R	
71.4	5	S		68.1	194	S	
100	7	total		100	285	total	
2.2	1	I	Oxacillin	0	0	S	Cefoxitin
54.3	25	R		0	0	total	
43.5	20	S		3.8	8	I	Ceftazidime
100	46	total		15.5	33	R	
2.2	1	I	Penicillin	80.8	172	S	
39.1	18	R		100	213	total	
58.7	27	S		81.3	13	S	Ceftizoxime
100	46	total		18.8	3	R	
2.8	2	I	Vancomycin	100	16	total	
9.9	7	R		2.1	5	I	Ceftriaxone
87.3	62	S		18.5	45	R	
100	71	total		79.4	193	S	
				100	243	total	

Abbreviations; S: Sensitive, **I:** Intermediate, and **R:** Resistance.

Antibiotic susceptibility patterns of isolated Gram-positive and Gram-negative Bacteria are shown in Tables 3 and 4. Among Gram-positive bacteria, vancomycin (98.5% sensitivity), amoxiclav (96.6% sensitivity), gentamicin (95.5% sensitivity) and nitrofurantoin (95.3% sensitivity) can be found in *S. epidermidis* cultures. Due to the high sensitivity of this bacterium to these antibiotics, it was used as a selective antibiotic. Of course, amoxicillin also had 100% sensitivity, but it was evaluated in only one sample. Also, in positive cultures of *S. saprophyticus*, the highest sensitivity is related to gentamicin (92.7% sensitivity).

The results showed that amikacin can be used for *P. aeruginosa* bacteria because 96.4% (53/55) were sensitive to amikacin. Amikacin was the most suitable choice in the samples, 757 samples of *E. coli* were evaluated for their sensitivity to amikacin, and 751 cases (99.2%) were sensitive to it. Also, sensitivity to amikacin in *P.aeruginosa* was 96.4%, in *Klebsiella* spp. was 97.8%, in *Enterobacter* spp. was 90.9%, in *P. mirabilis* and *Citrobacter* spp. was 100%, according to which it can be said that amikacin is the best choice when the answer of bacterial culture is not precisely defined.

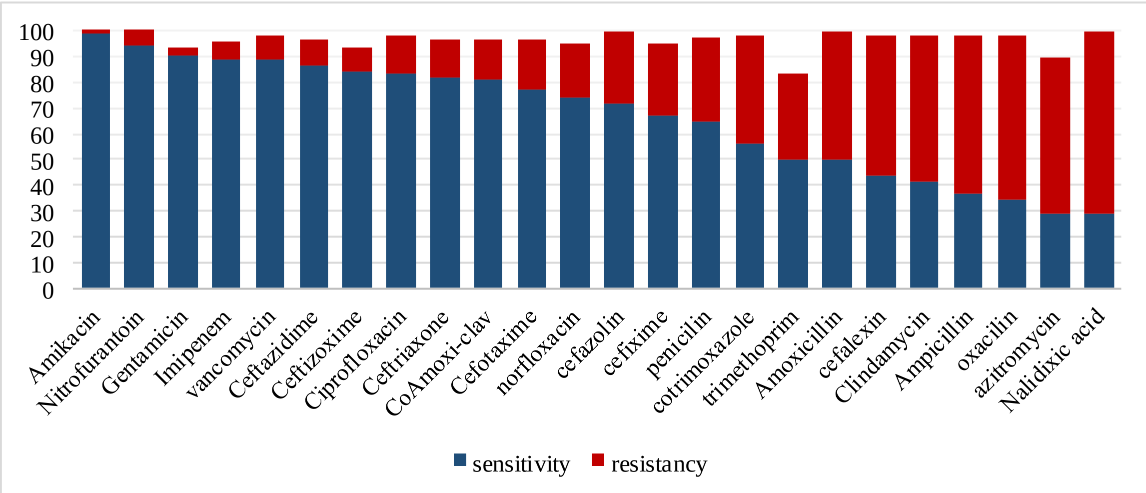

Figure 3: Antibiotic resistance pattern in all the positive cultures.

Table-3. Antibiotic Susceptibility Patterns (%) of Isolated Gram-negative Bacteria.

Organism	Amikacin	Amoxicillin-Clavulanate	Ampicillin	Cephalexin	Cefazolin	Cefixime	Cefotaxime	Ceftazidime	Ceftriaxone	Ciprofloxacin	Co-trimoxazole	Gentamicin	Imipenem	Nalidixic Acid	Nitrofurantoin
E .coli	99.2	82.5	37.6	53.5	59	69	82.4	89.7	88.9	84.2	84.1	92.7	77.8	37.5	96.2
P. aeruginosa	96.4	25	0	10	0	0	31.1	64.6	57.1	63.9	50	71.4	96.1	0	29.6
***Klebsiella* spp**	97.8	42.9	2.2	9.1	100	50	26.8	64.3	100	57.5	84.6	39.6	–	66.7	80
Proteus	100	40	25	100	80	100	60.9	84.2	75	57.9	81.8	92.3	100	–	73.1
***Enterobacter* spp**	90.9	75	10	11.1	66.7	33.3	73.7	80	100	73.7	88.9	87	–	0	95.7

- "–" indicates that the antibiotic was not tested or not applicable for that organism.

Table-4. Antibiotic Susceptibility Patterns (%) of most common Gram-positive Bacteria.

Organism	Amoxicillin-Clavulanate	Ampicillin	Azithromycin	Cephalexin	Cefazolin	Cefotaxime	Ceftriaxone	Ciprofloxacin	Clindamycin	Co-trimoxazole	Gentamicin	Nitrofurantoin	Oxacillin	Penicillin	Vancomycin
S. epidermidis	96.6	78.4	66.7	66.7	93.4	90.5	94	88.7	61.5	80.5	95.5	95.3	52.8	70.4	98.5
S. saprophyticus	75	52.2	22.2	66.7	84.6	71.4	79.3	84	8	48.9	92.7	88.6	3.3	44.4	61.7
S. aureus	100	57.1	0	–	25	40	63.3	66.7	25	63.6	83.4	77.8	0	55.6	90.9

- "–" indicates that the antibiotic was not tested or not applicable for that organism.

4- DISCUSSION

In the present study, the highest rate of UTI was observed in girls and in the age group above five years. The highest strain isolated from urine cultures was *E. coli* (54.80%), while the lowest strains belonged to *Acinetobacter*, *Streptococcus betahemolytic B*, and *E.faecalis* (0.07%). In agreement with our results, Duicu et al. found that *E. coli* is a common uropathogen in children hospitalized in the nephrology department, but the prevalence of *Klebsiella* in their study was higher than in ours (15.8% vs. 3.13%) (17). The frequency of infection with *E. coli* in our study was similar to studies in Nepal and Turkey, while the prevalence of the *Klebsiella* pathogen was lower than in those studies (18, 19).

E. coli exhibited the highest resistance to nalidixic acid (62.5%) and the highest susceptibility to amikacin (99.20%). Contrary to the results of our study, in previous study performed by Ranjbar et al., nitrofurantoin was reported as the most effective antibiotic with sensitivity (90%) (20). However, it should be noted that in our study, nitrofurantoin had a sensitivity percentage close to that of Ranjbar et al., but it was not the most sensitive antibiotic.

In this study, out of 1502 positive urine culture samples, 1034 cases (68.8%) were related to girls and 468 cases (31.2%) were related to boys, which is in line with the results obtained in the studies of Shahrkenya et al. in Birjand (21)., Qarashi et al. in Tabriz (22), Coban et al. (23), Mantadakis et al. (24) and Wakim-Hanna et al. (25).

In the current study, the highest rate of UTI infection was in children over 5 years of age, which could be due to exposure to more infection conditions such as being in swimming pools, public toilets. Conversely, Ranjbar et al. showed that the highest age of infection was children under two years of age. Of course, it should be noted that the sample size of this study included only 160 children compared to our study. In contrast to our study, a previous study demonstrated that the majority of the statistical population included children under 1 year old (17).

In our study of 1502 pediatric cases, the most common bacteria responsible for UTIs were *E. coli*, *S. epidermidis*, *P. aeruginosa*, *S. saprophyticus*, *Klebsiella*, and *P. mirabilis*. In almost all similar studies, *E. coli* was the most common cause of UTI (26-28). Although proportions vary by center and year, in our study, *E. coli* had the highest resistance to ampicillin (60.7%) and nalidixic acid (62.5%). *E. coli* showed the highest sensitivity to amikacin (99.2%) and nitrofurantoin (96.2%). In another study, the highest resistance was against cotrimoxazole with 64.6%, while we reported that 39.9% of samples were resistant to this antibiotic. In agreement with our study, Ranjbar et al. reported the highest sensitivity to nitrofurantoin and ciprofloxacin, which was 90% and 81.5%, respectively (20). Our previous study demonstrated that the most prevalent etiological agent of UTIs was *E. coli*, with the highest susceptibility to amikacin (29).

In the case of *Klebsiella*, antibiotic resistance to ampicillin was highest. 44 out of 45 samples were resistant to this antibiotic, and the highest sensitivity was to amikacin (97.8%). On the other hand, a more recent study (20) showed that cotrimoxazole (78.6%), cefixime (28.6%) and ceftazidime (71.4%) had the highest resistance, while ciprofloxacin (78.6%) and nitrofurantoin (71.4%) had the highest sensitivity.

In the present study, *P. aeruginosa* showed the highest susceptibility to amikacin. However, in contrast to our study, another investigation showed that *P. aeruginosa* had the highest resistance to cotrimoxazole and nalidixic acid (100%) (20).

As the frequency of antibiotic resistance varies regionally and over time, it is advisable to observe the pattern of antibiotic resistance. Resistance may decrease due to genetic factors, so conduct further studies in this area are necessary to reduce the use of antibiotic and their rational prescription by doctors. Raising public awareness of the dangers of indiscriminate antibiotics treatment may also help reduce bacterial resistance. It is also suggested that studies be conducted on a larger statistical population to obtain more accurate results.

This study only examined people admitted to the hospital. It is suggested to design a study to evaluate people who are treated on an outpatient basis. The evaluation of people who are hospitalized can also be affected by nosocomial infections, which should be considered as an effective factor in the results in future studies.

4-1. Limitation

This study has limitations as a retrospective analysis. Patient data such as demographic characteristics, clinical history, complications, ultrasound findings, and underlying conditions, were not consistently or comprehensively documented. Consequently, this limited the scope of detailed statistical and subgroup analyses. Therefore, prospective studies with standardized and comprehensive data collection are recommended to better evaluate risk factors and predisposing conditions in pediatric UTIs.

5- CONCLUSION

Antibiotic resistance is increasing due to the misuse or overuse of antibiotics worldwide. To prevent antibiotic resistance, the most appropriate narrow-spectrum antibiotic should be selected based on its susceptibility pattern.

According to the findings of this study, the use of amikacin, nitrofurantoin, gentamicin, imipenem, ceftazidime, vancomycin, and ceftriaxone antibiotics is recommended for the primary treatment of UTIs. However, in any case, the correct treatment for this type of infection first requires a detailed investigation of the drug resistance of the infectious agent and then prescribing medicine.

6-ACKNOWLEDGMENT

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7- FUNDING

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8-DECLARATIONS

8-1. Conflict of Interest

The authors declare no conflicts of interest.

8-2. Availability of Data and Materials

The datasets utilized and/or analyzed during the study are available upon reasonable request.

8-3. Ethics Approval and Consent to Participate

All experimental protocols were approved by the Ethics Committee of Mazandaran University of Medical Sciences (Ethical ID: IR.MAZUMC.REC.1402.686). Written informed consent forms were provided by the patients or a close relative before hospitalization, and the categorizing information of each sample was kept confidential.

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